AD_____

Award Number: W81XWH-11-1-0222

TITLE: Novel Pharmacological Targets for NF2-Mediated Yap/Taz Signaling

PRINCIPAL INVESTIGATOR: Morvarid Mohseni

CONTRACTING ORGANIZATION: Boston Children's Hospital
Boston, MA 02115

REPORT DATE: September 2012

TYPE OF REPORT: Annual summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
September 2012	Annual summary	1 September 2011 – 31 August 2012

4. TITLE AND SUBTITLE

Novel Pharmacological Targets for NF2-Mediated Yap/Taz Signaling

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-1-0222

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Morvarid Mohseni

E-Mail: mori.mohseni@me.com

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Boston Children's Hospital
Boston, MA 02115

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Mutations within the Nf2 gene, encoding the protein product Merlin, are the primary cause of NF2, however the precise mechanism by which Merlin promotes cellular proliferation and tumor formation is unclear. Recent studies have shown that Nf2-mediated cellular proliferation is a consequence of aberrant signaling of the Hippo pathway. Activation of the Hippo pathway via a series of phosphorylation events leads to the inactivation and phosphorylation of Yap, the downstream transcriptional coactivator of the TEAD family of transcription factors. In the absence of Merlin, Yap translocates to the nucleus where it binds to TEADs to promote pro-proliferative and anti-apoptotic programs. Yap and Merlin interact indirectly, however the direct binding partner(s) linking Yap and Merlin have not been discovered to date. Thus, the identification of putative binding partners and novel downstream targets of Nf2-mediated Hippo pathway activation is of interest. The primary objective of this proposal was to identify novel downstream targets of Nf2-mediated cell proliferation in the context of Hippo signaling. Since Yap is a downstream target of Merlin, I plan to identify therapeutic targets that lie between the Merlin and Yap signaling cascade that could result in the inhibition Merlin-mediated cell proliferation using a reporter-based high-throughput genome-wide RNAi screen in a human schannoma cell line, HEI-193. Since the TEAD family of transcription factors are essential in mediating Yap-dependent gene expression we developed DNA constructs that carry multimerized copies of a TEAD-DNA binding site (TBS) upstream of a promoter driving the expression of luciferase or mCherry reporter. The dynamic range of the reporter indicates that we are able to reliably reproduce a 15-fold induction of the reporter by transfection of constitutively active Yap1 (YapS127A) or siRNA knockdown of NF2.

15. SUBJECT TERMS
None provided.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	7	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

1. INTRODUCTION:

Neurofibromatosis type II (NF2), is an autosomal dominant genetic disorder that is characterized by the development of symmetrically distributed vestibular schwannomas as well as gliomas such as meningiomas and ependymomas. Since tumors of NF2 patients are slow-growing, chemotherapy treatment is not effective, thus traditionally patients must undergo surgical removal of tumors and radiation therapy. Sadly, these procedures are often performed multiple times over a lifetime due to tumor relapse, thus the identification of novel and specific pharmacological drug targets for the treatment of NF2 is a high priority. NF2 is due to mutations residing in the Nf2 gene that encodes the protein product Merlin. The precise mechanism by which Merlin promotes cellular proliferation and tumor formation is unclear. However, recent studies have implicated the hippo-signaling pathway as being the primary mechanism for proliferation in cells that lack or express mutant Nf2. Additionally, there is evidence demonstrating that a known hippo-component, Yap, indirectly binds to NF2 in the cell, however, the precise nature of these biochemical interactions is unknown. Since Yap is a downstream target of Merlin, I plan to identify specific therapeutic targets that can inhibit Nf2-mediated tumor growth in the context of Hippo-signaling. To do this, I will be making use of reporter cell system developed in my lab and a high throughput screen performed in collaboration with Harvard Medical School. The screen will allow for me to systematically knockdown each gene in the human genome, and identify unique genes that are specifically responsible for Nf2 mediated Hippo pathway activation. Furthermore, the identification of these novel genes may allow for a new focus on pharmacological targeting and therapeutic treatments for NF2.

2. KEYWORDS:

Hippo, NF2, Yap1, Taz, TEAD, RNAi, Merlin, Neurofibromatosis, Organ Size, Tumorigenesis, Reporter, Screening.

3. OVERALL PROJECT SUMMARY:

Current Objectives: Develop TEAD-reporter cell line that is responsive to NF2-loss, highly reproducible and can be utilized for high throughput screening.

Results: Several cell lines during the reporting period have been developed, including HEI-193 TBS-mCherry, HEI-193 TBS-Luciferase, HaCaT-TBS-Luciferase. These reporter cell lines were fully characterized in terms of growth, levels of reporter activity, responsiveness to NF2 loss and effective dynamic range. Following infection and selection of the cell lines for ones carrying a copy of the TEAD-reporter, clonal cell lines went on to be further evaluated. Characterization of the monoclonal TEAD-reporter lines (TBS-mCherry, TBS-luc) was performed by initially testing growth kinetics compared to the parental cell lines using standard MTS-assays. Once several cell clones were identified that displayed

similar growth kinetics to the parental controls, they were then tested for responsiveness to NF2-loss. In the absence of NF2, the TBS-reporter becomes active and one should visualize an increase in mCherry fluorescence or an increase in bioluminescence for the TBS-luciferase lines. Initial testing of 35 clones from each cell line resulted in 10 clones that have a robust increase in reporter activation. The remaining clones also displayed substantial fold-changes of TBS-activation over the control, but we limited it to the top 10 clones for simplicity. These remaining clones were further tested for their dynamic range by RNAi knockdown of Yap1, Taz and TEAD1. This was performed under two different contexts. The first context was to understand what the baseline levels of Hippo activity are in these cell lines by knocking town TEAD, YAP and TAZ and comparing to the non-transfected lines. These data indicated to us that there is a basal level of TBS-activity, and by knocking down either Yap1, Taz, or TEAD1 we can further reduce this activity by 5-fold (vs. the untreated controls). The second context was to understand how far we can suppress the activation of the reporter by artificially activated it using siRNA's targeting NF2. By simultaneous knockdown of NF2 and Yap1, Taz, or TEAD1 we are able to reduce the activity of the reporter by approximately 15-fold over the control (knockdown of NF2 alone).

4. KEY RESEARCH ACCOMPLISHMENTS:

Tools (Reporter plasmids and highly sensitive cell lines) have been generated for the Neurofibromatosis research community to identify and subsequently validate the impact of NF2-specific therapeutics on the Hippo signaling pathway.

5. CONCLUSION:

The initial goals of this CDMRP-award were to make use of an existing human schwannoma cell-line (HEI-193) for a high throughout RNAi screen to identify novel genes that could selectively inhibit NF2-mediated Yap1 activation.

During the duration of this grant, I successfully published a first author paper (**Cell,** Volume 144, Issue 5, 782-795, 4 March 2011) identifying that cell-cell contacts are responsible for Yap1 activation. It is widely accepted that the gene, NF2, exists in cell-cell contacts, however, its precise role at these sites is not well understood.

It was following this that I embarked on a genome-wide RNAi screen (~23,000 genes) to identify therapeutic targets of NF2-mediated Yap1 activation. The rationale for this was to identify a signaling pathway that we could selectively inhibit using known small molecule inhibitors. The genome wide RNAi screen revealed approximately 150 genes that act downstream of NF2, that in their absence or inhibition results in at least a 50% reduction in

Yap1 activity. I have also identified a handful of genes (Zinc finger nucleases and proteases) that would be considered the strongest downstream therapeutic targets for NF2-mediated Yap1 activation. These are genes that we are currently pursuing in the lab to better understand the biochemical relationship between these genes and NF2.
Using this knowledge, we have set up the small molecule screen containing both positive and negative controls. Fortunately, we were able to identify a potent inhibitor of NF2-mediated Yap1 activation, Okadaic Acid, however, the target, PP2A, is quite promiscuous and not selective to NF2-signaling, as such, this gene is not well suited as a therapeutic target.

I have created HEI-193 cells that contain the TBS-mCherry and TBS-Luciferase reporter, additionally; I have created HEI-193 cells that also now express NF2. Both of these cell lines are used for the small molecule screen, firstly to identify potent inhibitors of NF2-mediated Yap1-activity, and secondly, to identify inhibitors that could potentially be Yap1-agonists. Using the data I obtained from the genome-wide RNAi screen, it became apparent that inhibitors of MAPK/JNK signaling results in enhanced Yap1 activity. It is possible that using agonists of this pathway may selectively inhibit NF2-mediated Yap1 activity. I have so far been unsuccessful at obtaining the selective MLK1 inhibitor, CEP-1347 from Cephalon Inc. to test this hypothesis.

I am currently in the screening phase of this project, and will likely have data in the next 6-weeks. However, much information was garnered from the genome-wide siRNA screen, and I am currently in the process of following up on several *novel* genes of interest that could potentially serve as a starting point for developing new selective therapeutics for NF2.
We have currently bred NF2 fl/fl (conditional-null) mice to Yap1 fl/fl (conditional-null), and we are now in the process of crossing these mice to P0-cre to identify if Yap1 deletion can prevent schwannoma formation.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

Mohseni M*, Schlegelmilch K*, Kirak O, Pruszak J, Rodriguez JR, Zhou D, Kreger BT, Vasioukhin V, Avruch J, Brummelkamp TR, Camargo FD. Yap1 acts downstream of α-catenin to control epidermal proliferation**. Cell.** 2011 Mar 4;144(5):782-95. doi: 10.1016/j.cell.2011.02.031. PubMed PMID: 21376238 *co-first authors

7. INVENTIONS, PATENTS AND LICENSES: Nothing to report

8. REPORTABLE OUTCOMES: Nothing to report

9. OTHER ACHIEVEMENTS: Nothing to report

10. REFERENCES:

Zhang N, Bai H, David KK, Dong J, Zheng Y, Cai J, Giovannini M, Liu P, Anders RA, Pan D. The Merlin/NF2 tumor suppressor functions through the YAP oncoprotein to regulate tissue homeostasis in mammals. Dev Cell. 2010 Jul 20;19(1):27-38.

Stamenkovic I, Yu Q. Merlin, a "magic" linker between extracellular cues and intracellular signaling pathways that regulate cell motility, proliferation, and survival. Curr Protein Pept Sci. 2010 Sep;11(6):471-84. Review.

Striedinger K, VandenBerg SR, Baia GS, McDermott MW, Gutmann DH, Lal A. The neurofibromatosis 2 tumor suppressor gene product, merlin, regulates human meningioma cell growth by signaling through YAP. Neoplasia. 2008 Nov;10(11):1204-12.

Hamaratoglu F, Willecke M, Kango-Singh M, Nolo R, Hyun E, Tao C, Jafar-Nejad H, Halder G. The tumour-suppressor genes NF2/Merlin and Expanded act through Hippo signalling to regulate cell proliferation and apoptosis. Nat Cell Biol. 2006 Jan;8(1):27-36. Epub 2005 Dec 11.

11. Appendices: Nothing to report